REMARKS

In this Amendment, claims 2-3, 5-8, 10-13, 21 and 23-27 are amended, and claims 9, 22, 28 and 29 are canceled. Thus, after entry of this Amendment, claims 2-8, 10-14, 21 and 23-27 will be pending in the application.

In order to facilitate examination of this application, the claims have been amended to recite the competition assay format (independent claim 21), embodiments where the first ligand is a protein phosphatase, and embodiments where the presence of the second ligand is determined in the bound fraction. These embodiments were previously recited in the claims.

Applicants reserve the right to pursue other embodiments of the invention in one or more divisional applications. Such other embodiments include: sandwich assay formats (independent claim 22), embodiments where the second ligand is a protein phosphatase, and embodiments where the presence of the second ligand is determined in the non-bound fraction.

In addition, typographical errors have been corrected in claims 3 and 12. Typographical errors on page 9 of the specification have also been corrected.

No new matter has been introduced.

Entry of this Amendment is respectfully requested.

I. Telephonic Interview

Initially, Applicants representative, on behalf of the Applicants, would like to thank the Examiner for the helpful discussion of this application during the telephonic interview conducted on November 9, 2005. The required Statement of Substance of Interview is being filed herewith.

II. Response to Claim Rejections Under 35 USC §112, Second Paragraph

At page 2 of the Office Action, the Examiner rejects claims 2-14 and 21-29 as being indefinite for the following reasons.

(1) The Examiner contends that claims 21 and 22 omit an essential step, namely the step that details how the second ligand is determined.

Claim 22 has been canceled without prejudice or disclaimer.

The rejection of claim 21 is respectfully traversed.

The breadth of a limitation should not be confused with indefiniteness. MPEP §2173.04. In independent claim 21, the step of "determining" the presence of the second ligand ***is not*** indefinite. The fact that no particular detection method is specifically recited in the claim merely leaves the claims generic to specific methods of detection, and ***is not*** an omission of an essential step. Exemplary methods of detection are described at pages 8 through 10 of the specification, for example.

(2) The Examiner contends that the limitation "wherein the presence of said second ligand in either the bound fraction or the non-bound fraction is indicative of the presence of said phosphatase targeting toxin in said sample" is indefinite. The Examiner states that the second ligand would have to be in either the bound fraction or the non-bound fraction, regardless of whether toxin was present in the sample.

Amended independent claim 21 recites: "wherein the amount of said second ligand ***in the bound fraction*** is indicative of the amount of said phosphatase targeting toxin in said sample."

(3) The Examiner contends that claims 9 and 10 are indefinite because it is not clear to the Examiner how a reporter moiety on the first ligand would aid in detection of the toxin.

Claim 9 is canceled.

Claim 10 recites that the ***second ligand*** carries a reporter moiety, and thus, the rejection of claim 10 as indefinite is respectfully traversed.

(4) The Examiner contends that claim 29 is indefinite, because it is unclear to the Examiner how the first and second ligands would interact if both were protein phosphatase enzymes.

Claim 29 is canceled.

It is believed that the amended claims fully comply with section 112, second paragraph, and accordingly, withdrawal of these rejections is requested.

III. Response to Section 103 Rejections

(1) At page 3 of the Office Action, the Examiner rejects claims 22, 2, 3, 5, 6, 8, 9, 10, 13, 14, 24, and 26 under 35 USC §103(a) as being obvious over Holmes (US Patent 5,180,665) in view of Maggio (Enzymes as Immunochemical Labels).

Specifically, the Examiner contends that Holmes teaches a method of assaying for the presence of DSP toxins, comprising the steps of preparing and fractionating a marine extract, and contacting the fractions with a labeled substrate for protein phosphatase and at least one protein phosphatase enzyme. The Examiner also refers to column 3, lines 57-63 of Holmes stating that, without an appropriate separation technique, it is very difficult to conclude with certainty that the enzymatic activity of the protein phosphatase is inhibited by okadaic acid from the sample.

The Examiner admits that Holmes does not teach the use of ligands immobilized to a solid support.

However, the Examiner contends that Maggio teaches enzyme-immunoassays, which may involve a physical separation of the free and antibody-bound fractions. The Examiner believes that Maggio further teaches that, to maximize precision and sensitivity of an enzyme-immunoassay, one should seek complete separation of the free and bound fractions.

The Examiner concludes that it would have been obvious to a skilled artisan to modify the method of Holmes, based upon the teachings of Maggio, by using antibodies bound to a solid support to further separate the toxin from the extract.

Claim 22 has been canceled without prejudice or disclaimer.

With respect to independent claim 21, this claim has not been rejected over Maggio in view of Holmes, and thus it is believed that this rejection is now moot.

Nevertheless, it is believed that neither Maggio nor Holmes, alone or in combination, suggest ***immobilizing a protein phosphatase to a support*** for the determination of toxin in a sample via a ***binding assay***, as is recited in claim 21.

In addition, as provided by MPEP §2143.01, the proposed modification cannot change the principle of operation of a reference. Citing the decision of *In re Ratti,* 270 F.2d 810; 123 USPQ 349 (CCPA 1959), the MPEP states: the suggested combination cannot "require a substantial reconstruction and redesign of the elements shown in the primary reference as well as

a change in the basic principle under which the primary reference construction was designed to operate."

In the presently cited references, the assay of Holmes is an enzymatic assay while the assay of Maggio is a binding assay. More specifically, Holmes teaches measuring okadaic acid in a sample via its inhibitory effect on phosphatase enzymatic activity. Maggio, on the other hand, teaches immunoassays which detect the presence of components via antibody binding (which is not an enzymatic activity). The teachings of Maggio and Holmes operate on unrelated principles, and thus cannot be combined without a complete reconstruction of the Holmes assay, or without changing the fundamental principle upon which the Holmes assay is based (measuring phosphatase activity).

Accordingly, withdrawal of this rejection is requested.

(2) At page 5 of the Office Action, the Examiner rejects claims 21, 2-5, 8-10, 14, and 25-28 under 35 USC §103(a) as being obvious over Sikorska et al (US Patent 5,264,556), in view of Holmes.[1]

With respect to independent claim 21, the Examiner contends that Sikorska teaches an assay where free okadaic acid competes with an anti-idiotypic antibody for binding sites on immobilized antibody directed to okadaic acid.

[1] The Examiner acknowledged in the telephonic interview of November 9, 2005, that a section 103 rejection was intended, and not a section 102 rejection as set forth in the Office Action. Further, it is clear from the content of the rejection that it was intended to be a section 103 rejection.

The Examiner admits that Sikorska does not teach using protein phosphatase enzymes as ligands.

However, the Examiner contends that Holmes teaches that okadaic acid can be biologically inactivated by a single methyl esterification and therefore, procedures involving antibodies may still require validation by activity-based analysis.

The Examiner concludes that it would have been obvious to perform the method of Sikorska, as modified to use a protein phosphatase enzyme instead of an antibody, as allegedly suggested by Holmes.

This rejection is respectfully traversed, because ***neither Sikorska nor Holmes teach or suggest a protein phosphatase immobilized to a solid support, as is recited in amended independent claim 21.***

Further, and for the reasons that follow, it is strongly believed that there is no motivation or suggestion in the prior art to combine the teachings of Sikorska with the teachings of Holmes.

As the Examiner is no doubt aware, to establish a *prima facie* case of obviousness, the prior art must suggest the desirability of the claimed invention. In this regard, the Examiner must consider the entire teachings of the references, and consider them in context, to avoid improper hindsight reconstruction of the present invention. See generally, MPEP §2143.01. Further, the Examiner is reminded that the Examiner's proposed modification of the references may not change the basic principle under which the primary reference was designed to operate. See *supra*.

(a) The teachings of Sikorska and Holmes are incompatible

At column 2, lines 1-8, Sikorska states that assays that employ liquid chromatography are sensitive but require sophisticated, expensive equipment, and are therefore not useful for field testing.

In comparison, the assay of Holmes ***requires*** fractionation by methods such as liquid chromatography (see col. 3 and claims 1 and 5 of Holmes).

Given the negative discussion of liquid chromatography by Sikorska, the skilled person would not combine the teachings of Sikorska with the teachings of Holmes, because not only does Holmes relate to an enzymatic assay rather than a binding assay, but also because ***Holmes teaches an assay that Sikorska specifically teaches the skilled artisan not to use***.

(b) Sikorska motivates the skilled artisan to employ antibody reagents, because these can be inexpensively produced using hybridoma technology

The entire focus of Sikorska is on the development of an anti-idotypic antibody that can mimic okadaic acid, and the use of this anti-idotypic antibody in detecting okadaic acid (see the abstract and column 2, lines 40-64).

The introductory section of Sikorska discusses prior art ELISA assays that use an antibody to okadaic acid and solid-phase bound okadaic acid as reagents (col. 2, lines 9-23). Sikorska criticizes these assays because the okadaic acid reagent must be extracted from sponges, and therefore its preparation is tedious and costly (see col. 2, lines 9-24 and col. 5, lines

21-31 of Sikorska). As a result, Sikorska indicates that such kits are unmarketable due to their high cost and complicated sample preparation.

Sikorska teaches that the solution to this problem is the development of an anti-idiotypic antibody to mimic okadaic acid, which can therefore be used as a substitute for the okadaic acid reagent. In fact, column 15, lines 14-17 of Sikorska teach that an advantage of the anti-idiotypic antibody 1/59 is that it is inexpensive to produce because it is available in unlimited quantities as a hybridoma product.

Thus, when Sikorska is read in the proper context, Sikorska motivates the skilled artisan to employ ***antibody reagents***, because these can be readily and inexpensively produced using hybridoma technology.

However, in the Official Action the Examiner asserts that it would have been obvious to a skilled person in view of Sikorska to substitute the 6/50 antibody, which is also produced by a hybridoma (see point 5 of column 8), with a protein phosphatase enzyme. *There is in fact no basis for such a modification of Sikorska.*

Protein phosphatase enzymes, like okadaic acid, must be at least partially purified from a biological source involving more complex and expensive methods than monoclonal antibody production. Thus, if anything, Sikorska teaches away from the use of a protein phosphatase enzyme as a toxin binding ligand, because, in view of Sikorska, antibodies are most desirable.

(c) The enzymatic assay of Holmes is an alternative to the method of Sikorska, and thus a skilled artisan would accept the entire, unmodified, teachings of the Holmes reference

The Examiner believes that Holmes motivates a skilled artisan to modify the teachings of Sikorska, by replacing the antibody of Sikorska with a protein phosphatase. Specifically, the Examiner refers to a passage in Holmes that states that okadaic acid can be biologically inactivated by a single methyl esterification, and that since procedures involving antibodies give no information on the viable biological activity of a detected toxin, these still require validation by ***activity***-based methods of analysis.

According to the Examiner, since Holmes teaches that protein phosphatases are capable of detecting the biological activity of okadaic acid, it would have been obvious to use a protein phosphatase instead of the 6/50 antibody of Sikorska.

However, the enzymatic assay of Holmes *is an alternative* to the method of Sikorska, and thus a skilled artisan would accept the entire, unmodified teachings of Holmes. More specifically, Holmes makes it quite clear that the detection of biological activity of okadaic acid by a protein phosphatase is assayed by the ability of okadaic acid to inhibit the dephosphorylation of a labelled substrate by protein phosphatases, i.e. its ability to ***inhibit the activity*** of the protein phosphatase enzyme. There is no basis for modifying these teachings, to use a protein phosphatase immobilized to a support in a toxin-binding assay. Such is contrary to the teachings of Sikorska, and is not contemplated by Holmes. The Examiner appears to be engaging in improper hindsight reconstruction of the present invention.

It is therefore believed that the present claims are non-obvious over Sikorska in view of Holmes, and accordingly, withdrawal of this rejection is respectfully requested.

(3) At page 8 of the Office Action, the Examiner rejects claims 21, 22, 2-14, 23, and 25-28 under 35 USC §103(a) as being obvious over US Patent 5,525,476 to Matsuura in view of Holmes.[2]

Specifically, the Examiner contends that Matsuura teaches an antibody specific for okadaic acid immobilized on a support. The Examiner contends that Matsuura further teaches a competitive assay for detecting okadaic acid by using a labeled antigen, and teaches a noncompetitive method using an excess amount of a labeled secondary antibody.

The Examiner admits that Matsuura does not teach using protein phosphatase enzymes instead of antibodies.

However, the Examiner contends that Holmes teaches that okadaic acid can be biologically inactivated by a single methyl esterification and therefore, procedures involving antibodies may still require validation by ***activity***-based analysis.

The Examiner concludes that it would have been obvious to perform the method of Matsuura, as modified to use a protein phosphatase enzyme instead of an antibody, as suggested by Holmes.

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[2] The Examiner acknowledged in the telephonic interview of November 9, 2005 that a section 103 rejection was intended, and not a section 102 rejection as set forth in the Office Action. It is clear from the content of the rejection that it was intended to be a section 103 rejection.

This rejection is respectfully traversed, because ***neither Matsuura nor Holmes teach or suggest a protein phosphatase immobilized to a solid support, as is recited in amended independent claim 21.***

Further, and for the reasons that follow, it is strongly believed that there is no motivation or suggestion in the prior art to combine the teachings of Matsuura with the teachings of Holmes.

Matsuura focuses entirely on assays for detecting toxins ***using monoclonal antibodies as binding agents***. In particular, the focus of Matsuura is on a newly developed monoclonal antibody which is advantageous because of its resistance to organic solvent and its ability to recognize the three kinds of shellfish toxins (see abstract). The entire focus of Matsuura is on binding assays using ***antibodies***, and thus, there is absolutely no suggestion that the assays should be modified to use a protein phosphatase as a binding agent.

Further, an ordinary skilled artisan reading Matsuura would not combine these teachings with the teachings of Holmes, because Holmes has simply nothing to do with binding assays (as discussed *supra*), but instead teaches the desirability of ***enzymatic assays*** where the activity of a protein phosphatase enzyme is assessed.

It is believed that the present claims are non-obvious over Matsuura in view of Holmes, and accordingly, withdrawal of this rejection is respectfully requested.

IV. Advantages of the Present Invention

The Examiner is requested to note the following advantages of the present invention.

The solution-based enzymatic assays as described by Holmes are based on the ability of toxins such as okadaic acid to inhibit protein phosphatase activity. The presence of toxin is assessed by measuring the ability of a sample to inhibit dephosphorylation of a labelled substrate by a protein phosphatase enzyme (PPE).

As is acknowledged by Holmes at column 3, the enzymatic assays are highly susceptible to interfering factors. First, samples often contain endogenous phosphatases that can cleave ^{32}P from the labelled substrate, and provide false results. Second, other (non-toxin) compounds in the sample, for example, salts such as NaF, can inhibit protein phosphatase activity and give false results. Finally, other (non-labelled) compounds present in a sample can act as a substrate for the protein phosphatase enzyme and give rise to further false results.

On the other hand, the binding assay of the present invention does not suffer from these disadvantages.

The binding interaction determined in the presently claimed method is less affected by other components of the sample. This advantage of the present invention was unexpected over the teachings of the art, because it could not have been reasonably predicted that binding assays using a protein phosphatases would not suffer from the same disadvantages as the enzymatic activity assays.

Evidence that the assay of the present invention is less sensitive to compounds that can interfere with the enzymatic protein phosphatase assay is provided in Example 6 of the present specification. For example, the Table at page 19 of the specification shows that the competitive

binding assay of the present invention is significantly less susceptible to interference by ATP, inorganic phosphate, NaF, caseine and histones.

It is noted that Holmes, in an attempt to avoid such non-specific inhibition of the enzymatic activity assay, teaches the use of a laborious HPLC clean-up step that makes the method more complex and less user-friendly. (See col. 3, lines 51-68 of Holmes).

The binding assays of the present invention which measure the binding of toxin to a protein phosphatase enzyme, also display advantages over the antibody based assays such as Matsuura and Sikorska.

First, the present invention can detect all physiologically relevant toxins in the sample that that are capable of binding to the protein phosphatase, as opposed to antibodies to toxins that usually detect only certain toxins. The fact that the assay of the present invention can detect a large range of toxins is demonstrated in Example 5 of the specification.

Second, the present invention has the ability to detect physiologically relevant toxins, and exclude inactivated toxins, because of the use of the ***physiologically relevant*** protein phosphatase enzyme as a binding ligand. In this manner, the results of the assay more closely correlate to the toxicity of the sample.

Third, a skilled artisan would not have reasonably expected a sensitive assay component, such as a protein phosphatase enzyme, to be successfully immobilized to a solid support without affecting the stability of the protein phosphatase, or affecting the ability of the reagent to act as a binding ligand.

While it was well known that antibodies could be immobilized to solid supports, an ordinary skilled artisan ***would not have reasonably expected a protein phosphatase enzyme to be successfully immobilized in this manner and still remain functional.***

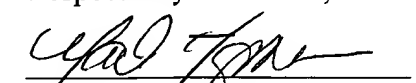
Fourth, the assays of the present invention are extremely sensitive, and can be used to detect pM amounts of toxin in water samples (see Example 7 of the specification). When used to detect toxins in shellfish extracts, the binding assays of the present invention were shown to be more sensitive than HPLC analysis (see Example 8).

IV. Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



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